



2016 Protocols

Terpene
Inhibition
Protocol

Purpose:

To test the inhibitory effects of some anti-microbial compounds found in pine tree oleoresin.

Required Materials:

- Pure α -pinene
- Terpenes extracted using steam distillation protocol
- Dimethylformamide (Sigma)
- *Escherichia coli* (DH5 α)
- *Saccharomyces cerevisiae*
- Luria-Bertani (LB) broth

Procedure:

1. Inoculate 1 DH5 α colony into 1 ml of LB broth and incubate overnight in a shaking incubator at 37°.
2. Next day, prepare serial dilution of α -pinene in Dimethylformamide (DMF).
 - a. In a 1.5 ml microcentrifuge tube add 50 μ l of DMF and 50 μ l of α -pinene.
 - b. Remove 50 μ l of DMF + α -pinene and add it to 50 μ l DMF.
 - c. Continue the dilution for a total of 8 dilutions.
 - d. Repeat this step for the Terpenes extracted using steam distillation
3. To 3 ml of LB, add 30 μ l of α -pinene + DMF, and 3 μ l of DH5 α .
4. Mix the solution well.
5. Incubate overnight in a 37° shaking incubator.
6. Observe dilutions the next day.

A DMF control was prepared containing 3 ml of LB, 3 μ l of DH5 α , and 30 μ l of DMF.

The same procedure was also conducted using *Saccharomyces cerevisiae*, instead of *Escherichia coli*.